

## Biological Activity of Cefotaxime Sodium and their Complexes with Iron and Copper Metals

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### Abstract

This investigation was designed to compare between inhibitory effect of cefotaxime sodium and their complexes with heavy metals (iron and copper). In this study, five concentrations were used in range from 0.01 M to 0.005 M for cefotaxime sodium and 0.005 mL, 0.01mL from FeCl<sub>3</sub> with 0.01mL cefotaxime sodium, and 0.005ml CuCl<sub>2</sub> with 0.01mL cefotaxime. The statistical analysis results showed that cefotaxime sodium (0.01mL) have a highest inhibition effect on bacterial growth, and iron have a synergistic effect with cefotaxime more than Copper. Also it was found that, E.coli species were recorded as most affected species by cefotaxime sodium, and their complexes relative to other species.

**Keywords:** Biological activity, Cefotaxime sodium, Heavy metals, Cefotaxime sodium, biological activity, E.coli.

### Introduction

A chemical structure of Cefotaxime sodium is 5-thia-1-azabicyclo [4, 2, 0] oct-2-ene-2-carboxylic acid, 3-[(acetyloxy) methyl]-[(z-amine-4-thiazoyl)

(methoxyimino) acetyl] amino]-8-oxo, monosodium salt, [6R-[6alpha, 7beta (2)]] This structure is shown in Figure 1.

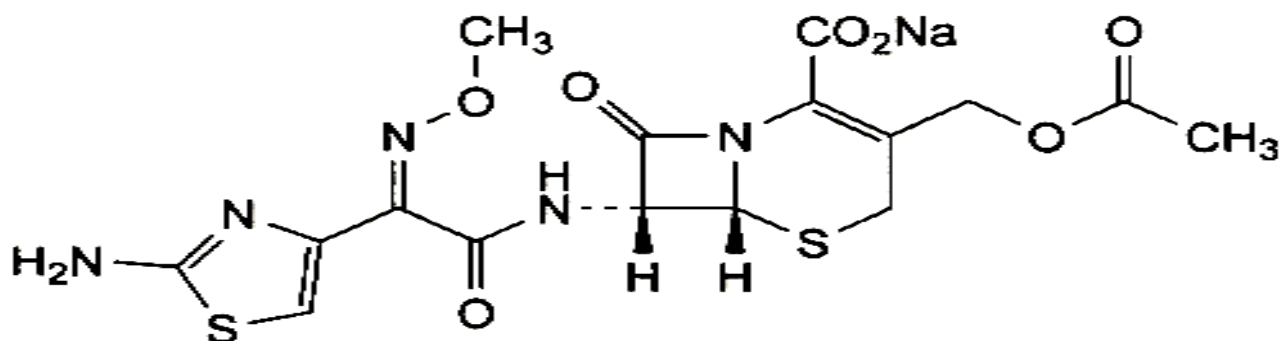


Figure 1: Description of chemical structure of Cefotaxime sodium

Bacterial resistance to heavy metals in the environment can through bioaccumulation, biotransformation, in ecological diversity, and co-selection of resistance factors for antibiotics<sup>1</sup> studied a toxicity of five elements to bacteria comprise the optimization of experimental parameters and the qualification of the metal concentrations across the inhibition zone. Biological activity

of Bo-1236, a new antipseudomonal Cephalosporin which showed potent activity against gram negative organisms in comparable to that of Ceftazidime, Cefotaxime, and Cefoperazone in susceptibility tests with clinical isolates. Also Bo-1236 showed protective activity superior to that of Ceftazidime, and Cefotaxime in experimental infection in mice caused by two

strain of *P. aeruginosa* and reveals activity in comparison to that of Ceftazidime and Cefotaxime against other gram negative bacterial infection. 2. 3 found three antibiotics (Cefotaxime, Carbenicillin, and Vancomycin) differ in their capacity to eliminate *Agro bacterium*, and Cefotaxime has been used in conjunction with Vancomycin to effectively eliminate bacteria when studied impacts of these antibiotics on *Chrysanthemum*, *Agro bacterium* and tobacco TCL morphogenesis.

More than 95 clinical isolates reportedly susceptible to Ceftazidime and Cefotaxime which was determined by Kirby-Bauer disc or by Minimal Inhibitory concentration (MIC) and KBPA Kirby-Bauer disc approximation method identified inducible phenotypes of third generation Cephalosporin resistance in 76% of isolates.[5], *In vitro* antimicrobial effect of Cefazolin and cefotaxime combined with minocycline against *Vibrio cholerae* non-01 non-0139 by using Minimal Inhibitory concentration (MICs) according to the agar dilution methods, and the results showed was inhibited firstly but resumed later when Cefazolin, Cefotaxime, or minocycline was used alone, but when Cefazolin or Cefotaxime was combined with minocycline, *Vibrio cholerae* was inhibited over 48hr and no re growth was noted, that's mean Synergistic effect.

## Material and Methods

### Chemicals

Cefotaxime sodium C<sub>16</sub> H<sub>16</sub> N<sub>5</sub> Na O<sub>7</sub> S<sub>2</sub> Which supplied from Lark Laboratories Ltd India. Ferric trichloride (Fe Cl<sub>3</sub>. 6H<sub>2</sub>O, 98%), it was provided by Fluka, Copper dichloride (CuCl<sub>2</sub>.2H<sub>2</sub>O, 98%) and it was provided by Fluka.

### Solution Preparation

Cefotaxime solution that used in this study was prepared as 0.01M by weight 0.4774 g and dissolved in 100 mL distilled water. Then, 0.1 M of FeCl<sub>3</sub> [3] was mixed by weight 1.6221 g and dissolved in 100 mL distilled water. 0.1 M from CuCl<sub>2</sub> by weight 1.3445 g and dissolved in 100 mL distilled water. 0.001M & 0.005 M was prepared from cefotaxime using dilution factor.

Then, Equal volumes were mixed from Iron & cefotaxime by 0.001 M for each one. The mixture was heated at 50-60 °C in a water

bath for 15 minute. The resulted residue was collected and then it was dried at 100 °C. Also Cefotaxime was mixed by 0.01 M with 0.01 M & 0.05 M of Iron and 0.05 M from Copper. The obtained concentrations were used as follow: 0.01 M Cefotaxime sodium, 0.005 M Cefotaxime sodium, 0.005 M FeCl<sub>3</sub> + 0.01 M Cefotaxime sodium, 0.01 M FeCl<sub>3</sub> + 0.01 M Cefotaxime sodium, and 0.005 M CuCl<sub>2</sub> + 0.01 M Cefotaxime sodium.

### Antibacterial Activity Assay

The assay depends on Conventional agar plate-diffusion method which was used to estimate the antibacterial activity of medical plant extract (5). Overnight stock culture of all the bacterial strains were prepared in nutrient broth, also used Macchoncky agar E.M.B agar (Eosin Methylene blue) & blood agar as selective media. Single volume (0.5) ml of stock culture of each of the bacterial strains were implanted and thoroughly mixed with nutrient agar at 45 °C in separated petridishes and allowed to solidify at room temperature.

In these agar plates, holes of 7 mm in diameter made using sterilized cork borer and filled in triplicate with fixed volume, two concentration of Cefotaxime sodium and three concentrations with their complexes. All pet dishes still in refrigerator for all solutions to diffuse in culture media, after a standing period of one hour, the plates were incubated at 37 °C for 18-24 h. the diameter of inhibition zone was measured by Vernier Calipers.

### Biochemical Test for Using Microorganisms

To confirm the results, biochemical test are used for *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus Sp.* according to [6].

## Results

### UV-visible and Infrared Spectrum

In this work, all UV - Vis measurements were recorded using UV-visible spectrophotometer (Cintra 5- GBC-Australia). From the ultraviolet and visible and infrared spectrums for the Cefotaxime and their complexes with iron and copper, that is shown in Figures 1, 2, 3 and 4. It was observed the clear changes in the intensities and positions of the absorption peaks, this due to the Complexity effect.

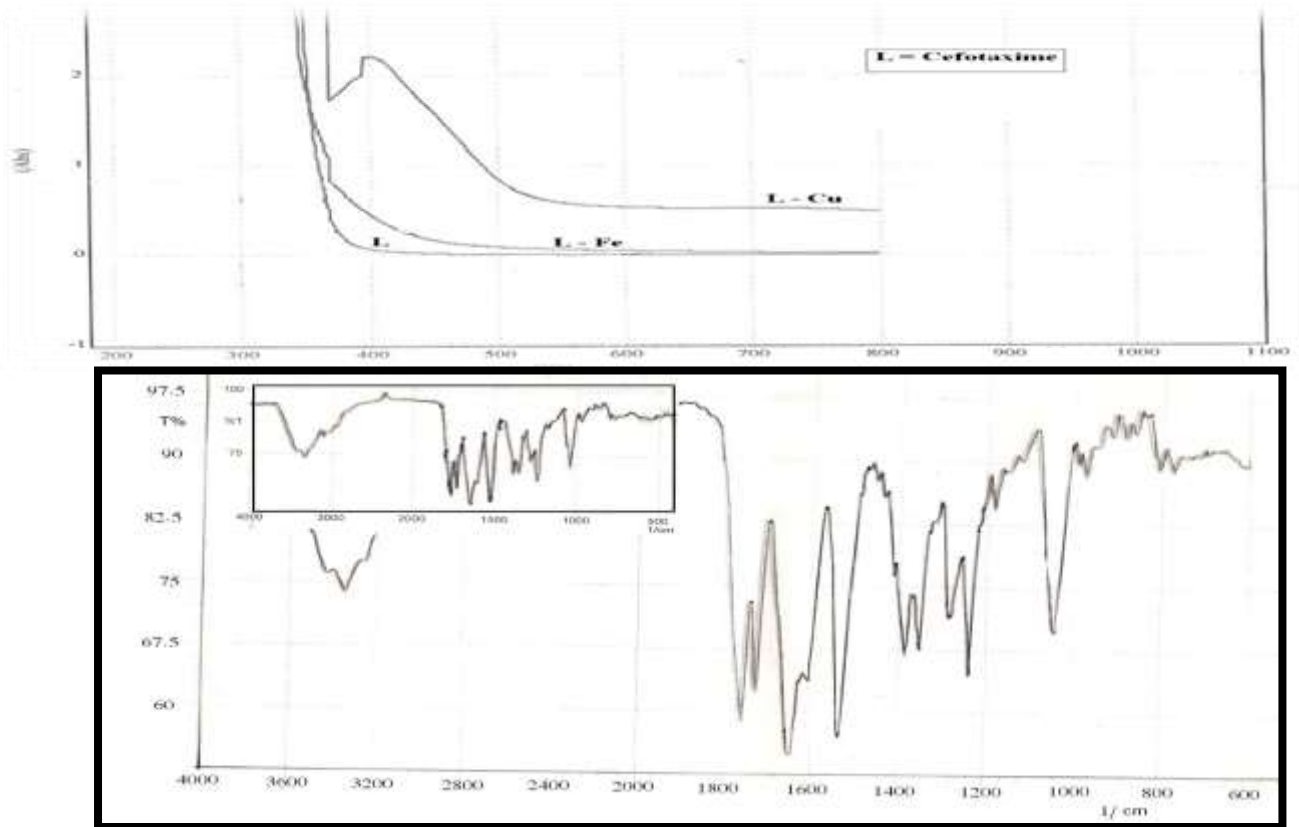


Figure 2: Infrared Spectrum for the Cefotaxime( $C_{16}H_{16}N_5NaO_7S_2$ )

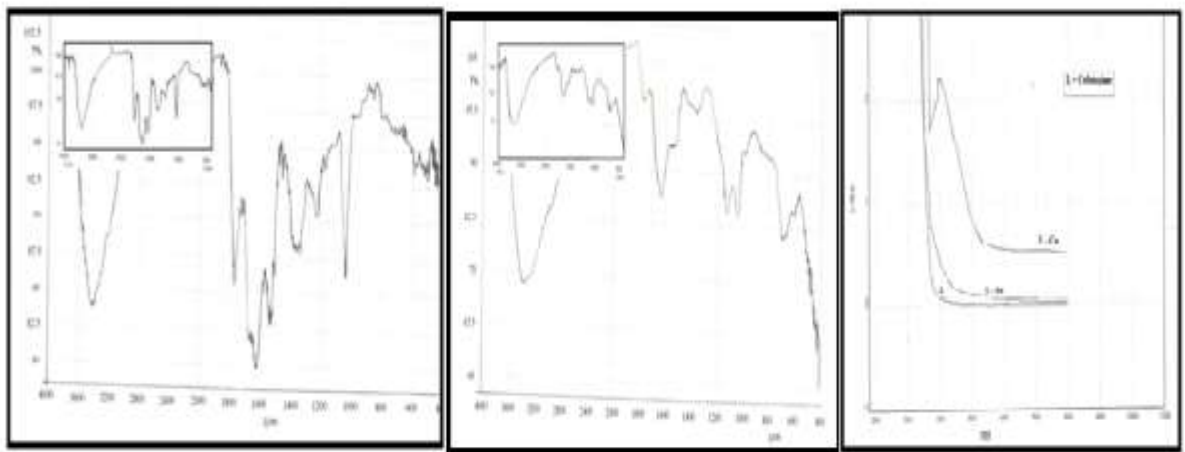


Figure 3: Infrared Spectrum for the complex of cefotaxime with Iron

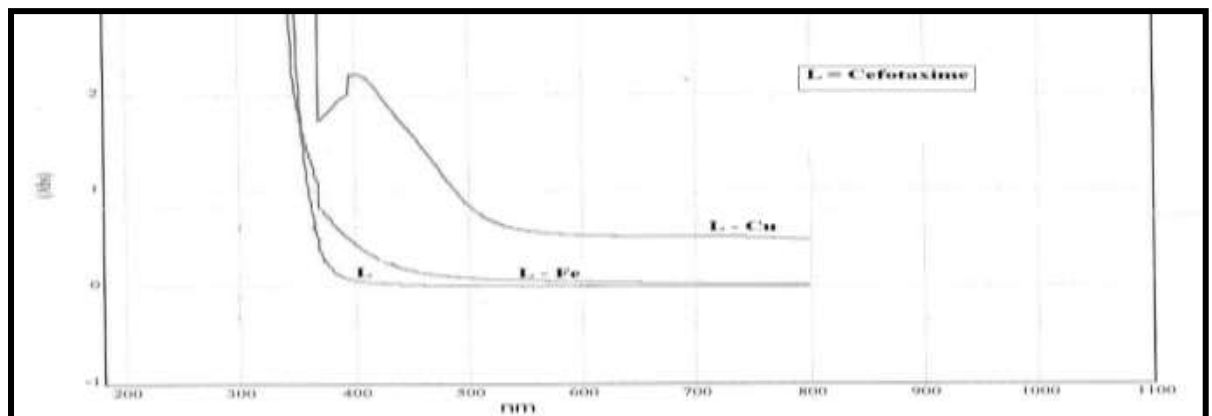


Figure 4: Infrared Spectrum for the complex of Cefotaxime with Copper

### Statistical Analysis

Results analyzed statistically by ANOVA test (Analysis of Variance) & revised least significant differences test (RLSD) through SPSS version 20.

### Results

Using Cefotaxime sodium at a concentration of 0.01 M This concentration has a wide spectrum of Inhibition effect on all studied species than another concentration with highest inhibition zone (2.17 cm) on E.coli species. The obtained results are shown in Table 1, and Figures 1 and 2. On the other hand, using Cefotaxime sodium with a concentration of 0.005 M A highest inhibition zone (2.13 cm) & lowest inhibition zone (1) were recorded for this concentration on E.coli & Klebsiella respectively as shown in Table 1, and Figure 1 and 3. Using a mixture of 0.005 M FeCl<sub>3</sub> + 0.01 M Cefotaxime sodium A

highest inhibition effect of this concentration on Klebsiella Sp. (1.3 cm) while a lowest effect on Staph.Sp. (0.5 cm).

These results are summarized in Table 1, and Figures 1 and 4. Using 0.01 M FeCl<sub>3</sub> + 0.01 M Cefotaxime sodium Streptococcus Sp Have most resistance species for this concentration with (0.5 cm) inhibition zone while (1.53 cm) represents a high inhibition zone on E.coli Sp. (Table 1, Figures 1 & 5). Using 0.005 M CuCl<sub>2</sub> + 0.01 M Cefotaxime sodium This concentration considered as a lowest inhibitory effect on Bacterial species with (1.8 cm ) & (0.6 cm ) on E.coli Sp. & Streptococcus Sp. Respectively ( Table 1, Figures 1 & 6 ).

E.coli is most effective species with all concentrations, and Streptococcus is lowest effective in comparison with another species.



Figure1: Inhibition zone of four types of Bacterial species by Cefotaxime sodium and their complexes

Table (1): Diameter of inhibition zone (cm) of bacterial growth for four species by cefotaxime sodium & their complexes.

Cefotaxime sodium & their complexes	Inhibition Zone diameter (cm )			
	<i>E.coli Sp.</i>	<i>Klebsiella Sp.</i>	<i>Staph Sp.</i>	<i>Strepto.Sp.</i>
1	2.17* ± 0.153	1.27± 1.8	1.37±1.9	1± 0.0
2	2.13 ± 0.774	1± 0.0	1.3±0.0	1.3± 0.5
3	1± 0.0	1.3 ± 0.58	0.5±1.0	1±0.0
4	1.53 ± 0.153	0.8 ± 0.1	1 ± 0.0	0.5±0.1
5	1.8± 0.59	0.8 ± 0.1	0.8± 0.1	0.6 ±1.0

p≤0.05

## Discussion

The bacterial development of antibiotics resistance is one of the best of documented examples of contemporary biological evolution after half a century of massive, largely uncontrolled release of industrial antibiotics around the world, microbial populations have developed a wide variety of mechanism of resistance. Carbencillin and cefotaxime have a broad spectrum of activity against both gram positive and gram negative organisms, and function by blocking the cell wall mucopeptide biosynthesis [3].

The ability of small amount of heavy metals to cause effectiveness on bacterial growth probably due to the high affinity of cellular proteins for the metallic ions, although the concentrations of ions in solution may be miniscule (a few part per million), cells die due to the accumulative effects for ions within the cell, and factors effecting microbial response to metals, the ways in which microbial activity may alter the metal balance of an environment and the

modifications produce in microbes by heavy metals ions [8], also a high concentration of iron and other trace elements could restricted bacterial growth and modify their metabolic pattern as well, and iron has inhibitory effects on bacterial growth at high concentration and Fe<sup>3+</sup> is also a toxic substance, also the toxic effects of the trace elements could support or remove in combination with other elements [9].

In addition, high level of iron plays an important role to inhibit the growth of bacteria and Fe (III) as well as Fe (II) has an inhibitory effect [9], a highly resistance of some organisms such as Staph aureus due to has multiple iron uptake systems, which may contribute to its ability to live in environments with variable concentration of iron [10]. In turns, antagonistic effect of copper with Cefotaxime sodium return to cells growing continuously in the presence of excess Cu (Cu adapted culture) which defines as cell actively growing in the presence of elevated Cu (approximately 6 h after inoculation of the culture).

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